BIOLOGICAL DIVERSITY AND CHEMICAL KNOWLEDGE AS DRIVING FORCES IN ENZYME ENGINEERING

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These Industrial Biotechnology holds the promise to manufacture chemical compounds based on efficient, green routes and using renewable substrates. Applying the tools of synthetic biology, novel artificial pathways are designed to access these chemicals. However, analyzing important target molecules with a retrosynthetic perspective we have to realize that we quite often lack enzymatic functions limiting this synthetic biochemistry approach. How do we get access to these sought-after enzymatic activities? In my talk, I will discuss grasping this challenge with a pinch from biology and chemistry.

Biologists provide us with structural information and sequence date for hundreds of closely related enzymes. Comparing these different enzymes, we can extract valuable information about structure function relationships. We can learn that nature has chosen certain hot spots in an enzyme to expand its substrate and reaction scope. Hot spots, insertions and deletions or sometimes correlated sides of amino acid variations, are an ideal starting point for designing small, focused libraries of enzyme variants. This strategy was successfully applied in engineering dioxygenases with an extended substrate spectrum as well as P450 monooxygenases with a higher activity

Identifying enzyme variants catalyzing new reactions is a bit more challenging. Moon shine activities or enzyme promiscuity has thought us that enzymes can do much more than converting physiological substrates. Under the order of the same catalytic mechanisms chemically related compounds can be converted and new type of reactions are performed by the very same enzyme. Even if the reactions are running with low rates, promiscuous substrates are the starting point for the development of enzymes with novel functions. Key to unravel possible reaction is the careful choice of the right substrates and calls for some chemical knowledge. This approach will be illustrated based on examples with squalene hopene cyclases catalyzing non-natural Prins-reactions, Friedel-Crafts-reaction, isomerizations or conversions.

Insights into the biological diversity of enzymes on one side and insights into the chemistry of enzymes enable us to engineer enzymes with novel activities in our laboratories. These new *bio*catalysts go far beyond what nature has intended to catalyze so far and thus expand our biosynthetic options. However, we also get a notion about the evolutionary options nature has built into these fantastic catalyst